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Cope

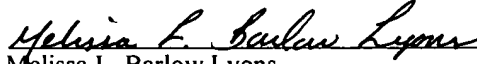
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Patentee: Ya Fang Liu  
Patent No.: 6,811,992 B1  
Confirmation No.: 9992  
Filed: September 17, 1998  
For: METHOD FOR IDENTIFYING JNK AND MLK INHIBITORS FOR  
TREATMENT OF NEUROLOGICAL CONDITIONS  
Examiner: Marianne P. Allen  
Art Unit: 1631

CERTIFICATE OF MAILING UNDER 37 C.F.R. §1.8(a)

The undersigned hereby certifies that this document is being placed in the United States mail with first-class postage attached, addressed to Mail Stop Certificate of Correction, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the 16<sup>th</sup> day of November, 2004.

  
Melissa L. Barlow Lyons

Mail Stop Certificate of Correction

Commissioner For Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

Sir:

Certificate  
NOV 23 2004  
of Correction

Transmitted herewith are the following documents:

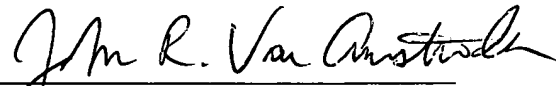
- ☒ Request for Entrance of Certificate of Correction Under 35 U.S.C. §254 & §255
- ☒ Certificate of Correction - Form PTO-1050
- ☒ Copy of pertinent pages from U.S. Patent No. US 6,811,992 B1
- ☒ Return Receipt Postcard

If the enclosed papers are considered incomplete, the Mail Room and/or the Application Branch is respectfully requested to contact the undersigned at (617) 646-8000, Boston, Massachusetts.

A check is not enclosed. If a fee is required, the Commissioner is hereby authorized to charge Deposit Account No. 23/2825. A duplicate of this sheet is enclosed.

Respectfully submitted,  
Ya Fang Liu, Patentee

By:

  
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Docket No.: L0624.70000US00  
Date: November 16, 2004  
xNDDx

-1 DEC 2004



DOCKET NO.: L0624.70000US00

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Patentee: Ya Fang Liu  
Patent No.: 6,911,992 B1  
Confirmation No.: 9992  
Filed: September 17, 1998  
For: METHOD FOR IDENTIFYING JNK AND MLK INHIBITORS FOR  
TREATMENT OF NEUROLOGICAL CONDITIONS  
Examiner: Marianne P. Allen  
Art Unit: 1631

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Melissa L. Barlow Lyons

**Mail Stop Certificate of Correction**

Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

REQUEST FOR ENTRANCE OF CERTIFICATE OF CORRECTION  
UNDER 35 U.S.C. §254 and §255

Sir/Madam:


Patentee respectfully requests the correction of two errors in the printing of the above-captioned patent. Specifically, the title "METHOD FOR IDENTIFYING MLK INHIBITORS FOR THE TREATMENT OF NEUROLOGICAL CONDITIONS" should be replaced with --METHOD FOR IDENTIFYING JNK AND MLK INHIBITORS FOR TREATMENT OF NEUROLOGICAL CONDITIONS--. Additionally, claim 4 has a typographical error made by the Patent Office. Please correct as follows: In column 23, line 57, "glutarnate" should be replaced with --glutamate--.

Patentee points out that the correction requested does not involve change in the patent that constitutes new matter or would require reexamination, and therefore, respectfully request that a certificate of correction be issued. Patentee encloses a copy of the pertinent pages of the issued patent with the errors highlighted. Since the error was made by the Patent Office, it is respectfully submitted that no fee is due. However, if the Examiner deems a fee necessary, the fee may be charged to Deposit Account No. 23/2825. Should any questions arise concerning the foregoing, please contact the undersigned at the telephone number listed below.

For the reasons stated above, Patentee respectfully requests entrance of the enclosed Certificate of Correction.

Respectfully submitted,  
Ya Fang Liu, Patentee

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Docket No. L0624.70000US00

Date: November 16, 2004

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UNITED STATES PATENT AND TRADEMARK OFFICE  
**CERTIFICATE OF CORRECTION**

PATENT NO. : US 6,811,992 B1  
DATED : November 16, 2004  
INVENTORS : Ya Fang Liu

It is certified that two errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On the title page, item (54) should read:

METHOD FOR IDENTIFYING JNK AND MLK INHIBITORS FOR TREATMENT OF  
NEUROLOGICAL CONDITIONS

In the claims:

**Claim 4,**

In Column 23, line 57, delete "glutarnate" and replace with --glutamate--.

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PATENT NO. US 6,811,992 B1

- 1 DEC 2004



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(12) **United States Patent**  
Liu

(10) Patent No.: **US 6,811,992 B1**  
(45) Date of Patent: **Nov. 2, 2004**

(54) **METHOD FOR IDENTIFYING MLK  
INHIBITORS FOR THE TREATMENT OF  
NEUROLOGICAL CONDITIONS**

WO WO 00/13015 3/2000  
WO WO 00/47583 8/2000  
WO WO 02/14536 2/2002

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(\*) Notice: Subject to any disclaimer, the term of this  
patent is extended or adjusted under 35  
U.S.C. 154(b) by 0 days.

(21) Appl. No.: 09/156,367

(22) Filed: Sep. 17, 1998

#### Related U.S. Application Data

(60) Provisional application No. 60/085,439, filed on May 14,  
1998.

(51) Int. Cl.<sup>7</sup> ..... C12Q 1/00; C12Q 1/68;  
C12Q 1/48; G01N 33/53; C12P 21/06

(52) U.S. Cl. .... 435/15; 435/4; 435/6;  
435/7.1; 435/69.1

(58) Field of Search ..... 435/4, 15, 6, 7.1,  
435/69.1

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(List continued on next page.)

*Primary Examiner*—Marianne P. Allen

(74) *Attorney, Agent, or Firm*—Wolf, Greenfield & Sacks,  
P.C.

(57) **ABSTRACT**

The present invention describes methods for identifying  
compounds that inhibit JNK and MLK kinase activity as  
drugs for treating a mammal susceptible to or having a  
neurological condition. This invention also discloses meth-  
ods for preventing neuronal cell death and treating neuro-  
logical conditions that involve neuronal cell death, particu-  
larly neurodegenerative diseases characterized by glutamine  
or kainate mediated toxicity, such as Huntington's disease  
and Alzheimer's disease.

13 Claims, 14 Drawing Sheets

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## Example 8

## 96-Well Cell Based Assay

HN33 cells (~60% of confluence), plated on a 96-well plate, were grown in DMEM-F12 medium supplemented with 10% of fetal bovine serum (FBS). Prior to transfection, the medium was removed and cells were washed with serum-free medium once and 50  $\mu$ l of DMEM-F12 medium with 1% of FBS was added. The full-length huntingtin expression plasmid containing 16 (control), 48 or 89 CAG repeats or lipofectin solution were diluted with HBS first and mixed 1x1 volume and the mixture was incubated at room temperature for 15 min. Ten  $\mu$ l of the DNA-lipofectin mixture was added to the culture medium. A mixture of compound from a chemical library was added 6 hours after transfection. Twelve hours after transfection, additional FBS was added to the final concentration of 10%. Forty-eight hours after transfection, cells were washed with PBS once and fixed with 4% paraformaldehyde dissolved in PBS and permeabilized with 1% Triton X100 in PBS containing 1% sodium citrate. Cells were then rinsed twice with PBS containing 1% BSA and cells were incubated with 25  $\mu$ l of TUNEL reaction solution for 1 hour at a 37° C. cell culture incubator. Cells were rinsed with PRS for three times and 25  $\mu$ l of converter-AP solution was added and cells are returned to the incubator and incubated for 30 min. Cells were rinsed three times with PBS with 1% BSA and cells were incubated in a BCIP solution for 1-5 min at room temperature and rinsed three times with PBS and analyzed under light microscope.

While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the spirit and scope of the invention as defined by the appended claims.

What is claimed is:

1. A method for assessing a compound's ability to prevent neuronal cell death, comprising:

a) contacting a compound with cultured neuronal cells having activated MLK activity, wherein activated MLK activity is selected from the group consisting of MLK1 activity, MLK2 activity, MLK3 activity, and wherein the activity is a kinase activity; and

b) determining the number of cultured neuronal cells that die; wherein a decreased number of dead cultured cells in the presence of the compound compared to the number of dead cultured neuronal cells in the absence of the compound is indicative of the compound's ability to prevent neuronal cell death.

2. The method of claim 1, wherein the neuronal cells are expressing a mutated protein selected from the group consisting of polyglutamine stretch-expanded huntingtin and C-terminal 100 amino acids of amyloid precursor protein, or the neuronal cells are treated with a neurotoxin to induce apoptosis.

3. The method of claim 2, wherein the neuronal cells are HN33 cells.

4. The method of claim 2, wherein the neurotoxin is glutamate, quinolinic acid or kainic acid.

5. The method of claim 1, wherein the neuronal cells are apoptotic neurons.

6. A method for assessing a compound's ability to prevent neuronal cell death, comprising:

a) contacting a compound with cultured neuronal cells expressing a mutated protein selected from the group consisting of polyglutamine stretch-expanded huntingtin and C-terminal 100 amino acids of amyloid precursor protein, or with neuronal cells treated with a neurotoxin to induce neuronal cell death; and

b) determining the number of cultured neuronal cells that die; wherein a decreased number of dead cultured neuronal cells in the presence of the compound compared to the number of dead cultured cells in the absence of the compound is indicative of the compound's ability to prevent neuronal cell death.

7. The method of claim 6, wherein the neuronal cells are HN33 cells.

8. A method for assessing the ability of a compound to prevent neuronal cell death, comprising:

a) contacting a compound with cultured neuronal cells having activated MLK activity, wherein the activated MLK activity is selected from the group consisting of MLK1 activity, MLK2 activity, MLK3 activity, and wherein the activity is a kinase activity;

b) contacting, in the presence of the compound, surviving cells from step (a) with an agent that induces apoptosis; and

c) comparing the level of apoptosis in the cells in the presence of the compound with the level of apoptosis in the cells in the absence of the compound; wherein the compound is a potentially useful drug for treating mammals when the level of apoptosis in the cells in the presence of the compound is less than the level of apoptosis in the cells in the absence of the compound.

9. The method of claim 8, wherein the apoptotic agent is a neurotoxin.

10. The method of claim 9, wherein the neurotoxin is glutamate, quinolinic acid or kainic acid.

11. The method of claim 8, wherein step (b) is performed by transfecting the surviving neuronal cells with nucleic acid encoding a mutated form of huntingtin or amyloid precursor protein.

12. The method of claim 8, wherein the neuronal cells are HN33 cells.

13. A method for assessing the ability of a compound to inhibit MLK activity and to prevent neuronal cell death, comprising the steps of:

a) contacting a compound with a MLK protein and a substrate therefor, wherein the MLK protein is selected from the group consisting of MLK1, MLK2, MLK3 and combinations thereof;

b) measuring the level of MLK activity, wherein the MLK activity is a kinase activity;

c) comparing the level of MLK activity in the presence of the compound with the level of MLK activity in the absence of the compound, wherein a decrease in MLK activity in the presence of the compound is indicative that the compound has an ability to inhibit MLK activity;

d) contacting the compound having an ability to inhibit MLK activity with cultured neuronal cells having activated MLK activity, wherein the activated MLK activity is kinase activity; and

e) comparing the occurrence of apoptosis in the cultured neuronal cells in the presence of the compound with the occurrence of apoptosis in the cultured neuronal cells in the absence of the compound; wherein the compound having an ability to inhibit MLK activity has the ability to prevent neuronal cell death when the occurrence of apoptosis in the cultured neuronal cells in the presence of the compound is less than the occurrence of apoptosis in the cultured neuronal cells in the absence of the compound.